Monitoring Fecal Indicator Bacteria with Alternative Real-Time PCR Instruments to Assess Health Risks Associated with Recreational Water Use

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US EPA guidance on the safety of surface waters for recreational use is currently based on epidemiological studies conducted in the 1980s that demonstrated a strong positive correlation between bathing-associated illness rates and concentrations of culturable fecal indicator bacteria in these waters. Culture-based methods for quantifying fecal indicator bacteria require at least 24 hours for results. Because swimmers may be exposed to unsafe waters during this time, attention is now shifting to molecular monitoring methods that generate results in the same day. A multi-year epidemiological study is in progress to determine the relationship between illness rates and concentrations of fecal bacteria in recreational waters as determined by real-time polymerase chain reaction (PCR) analysis. Analyses in the first two years of the study (2003–2004) were limited to one PCR reagent and type of instrument. Acceptance of this technology will be aided by the availability of choices in instruments and newer PCR reagents that offer even shorter analysis times. Studies were conducted to compare the performance of three instruments and associated reagent systems: Applied Biosystems model 7700 (analysis time ~2 hr), Cepheid Smart Cycler (analysis time ~ 30 min with TaqMan probe and ~45 min with Scorpion probe), and Applied Biosystems fast-block model 7900 (analysis time ~35 min), for the detection of two fecal bacteria groups, *Enterococcus* and Bacteroidetes, in surface waters. PCR probe and primer sets originally developed for detection of these organisms on the model 7700 gave less sensitive and precise measurements on the faster Smart Cycler and model 7900 instruments. Modifications of the PCR probe and primer sets improved the performance on these instruments. DNA extracts of 50 ml beach water filtrates (N = 396) collected from Biloxi, Mississippi, during the 2005 epidemiological study were analyzed on the three instruments using optimal probe and primer sets for each instrument. Mean *Enterococcus* and Bacteroidetes calibrator cell equivalents/filtrates determined from all analyses were 37 and 388 (coefficient of variation among instrument means: 0.26 and 0.24, respectively). Significant differences ($p < 0.05$) were found in the means for both groups of organisms between each of the systems with the exception of the Bacteroidetes results on the models 7700 and 7900. Normalization of results using reference control analyses made these differences non-significant in all comparisons except those involving the model 7700. Failure to show comparability between model 7700 and other systems' results may be related to the use of the different probe and primer sets. Analyses will be performed to determine the relationships between bathing-associated illness rates and measurements of both *Enterococcus* and Bacteroidetes on each of the three instruments. Results of these analyses will determine whether results from different real-time PCR instruments show the same positive correlation with illness rates that has been established thus far in the EPA epidemiological study. They will also provide additional data for the Office of Water to use in formulating new health risk-based water quality guidelines associated with this technology.